Inventor: CHEN et al.
Docket No.: 11669.0150USW2
Title: TRANSGENIC MICE EXPRESSING HUMAN CD20
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Human CD20 Transgene expression in mouse B220⁺ cells
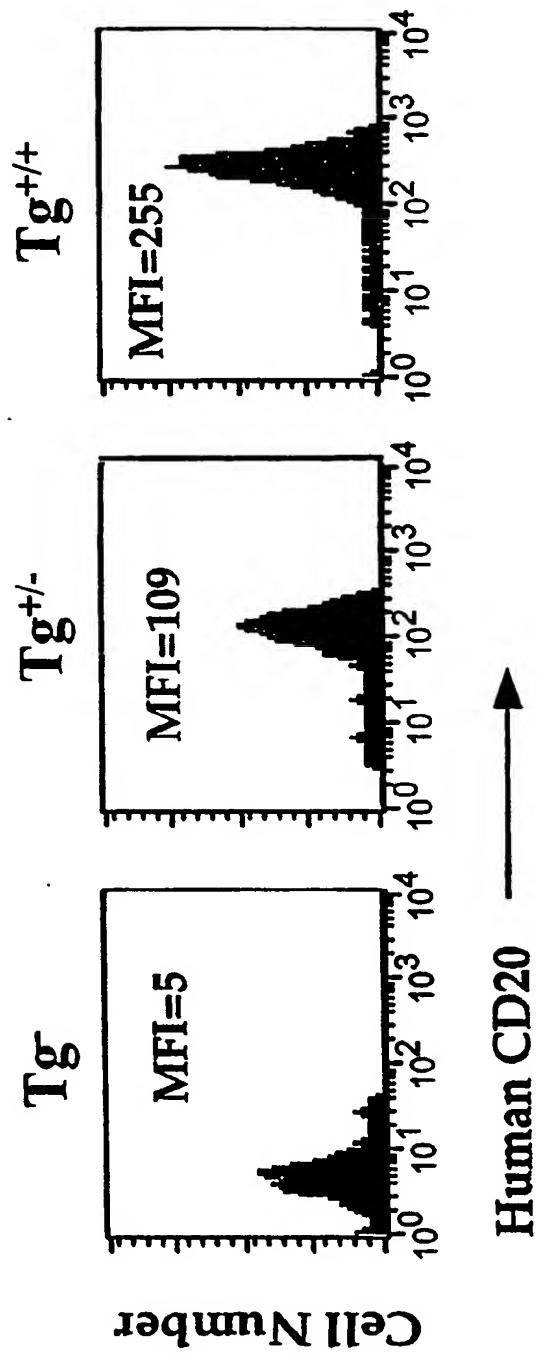
Figure 1
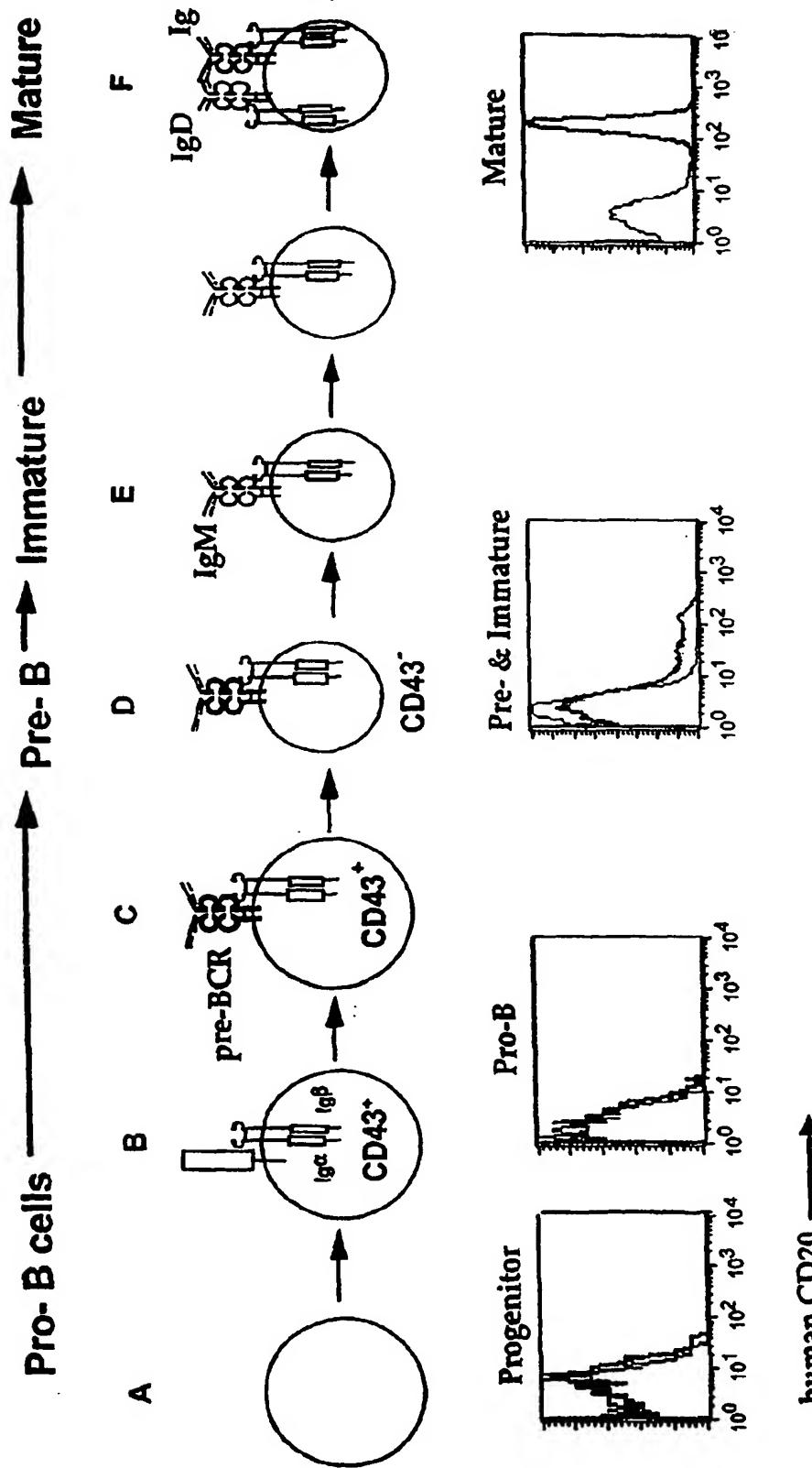
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Figure 2

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Expression of human CD20 in Tg+ mouse bone marrow
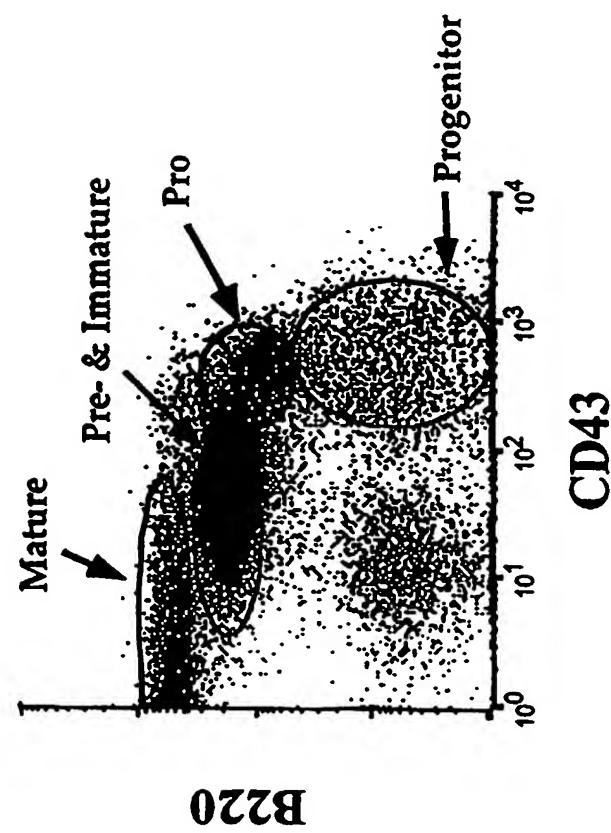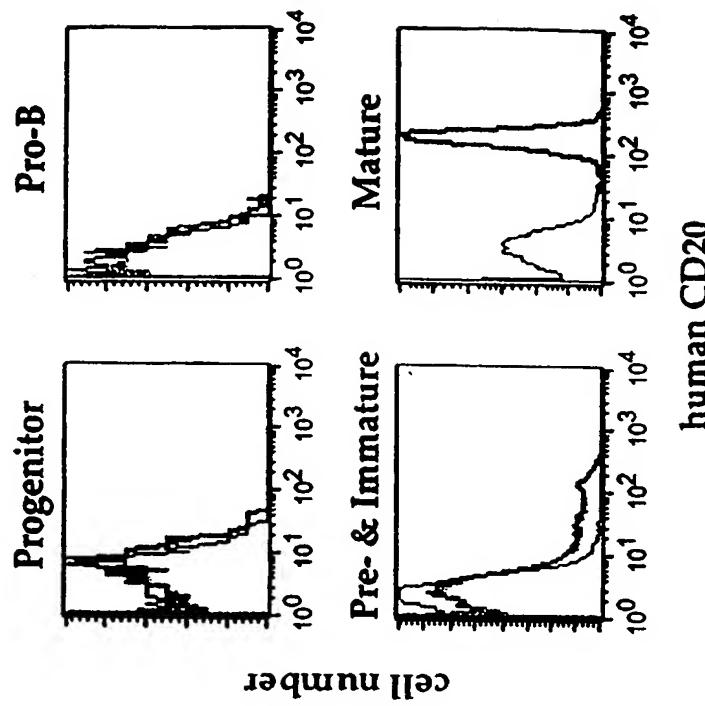
Figure 3

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Expression of human CD20 in Tg⁺ mouse splenic B cells

MFI	
	1033
	1201
	636

human CD20

Cell number

T2/Follicular

Marginal Zone

T1

B220+

CD21

IgM

Figure 4

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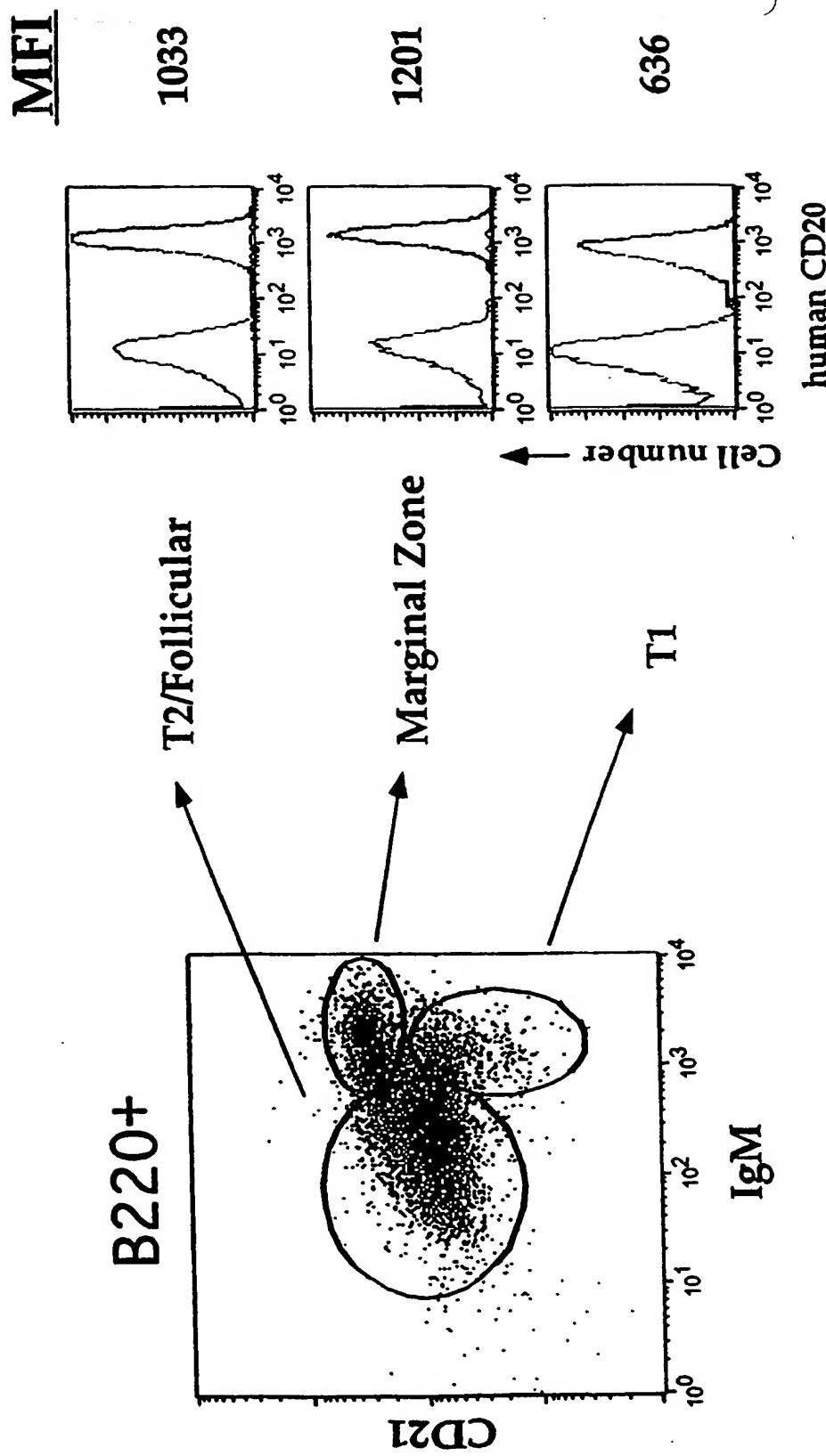
Expression of human CD20 in Tg$^+$ mesenteric LNs

Figure 5

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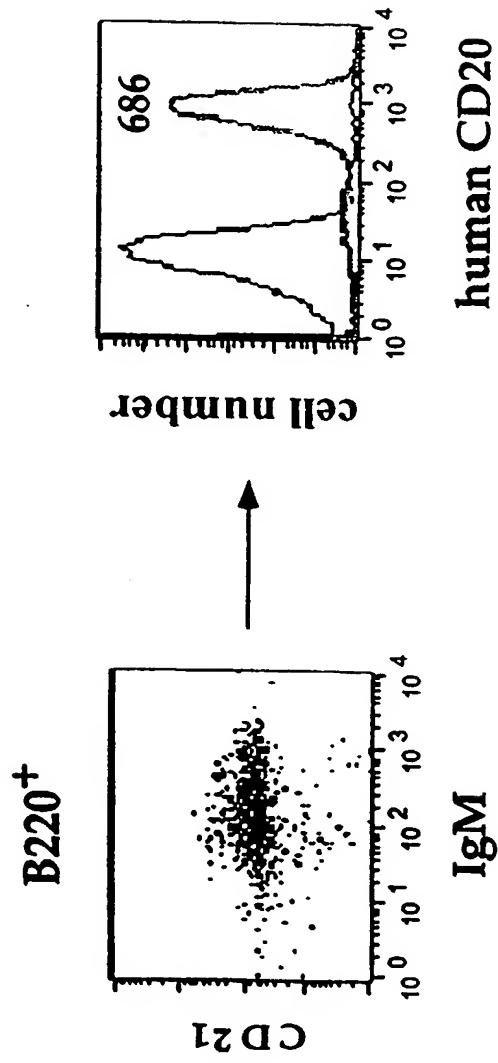
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Expression of human CD20 in Tg⁺ Peyer's Patches

Figure 6

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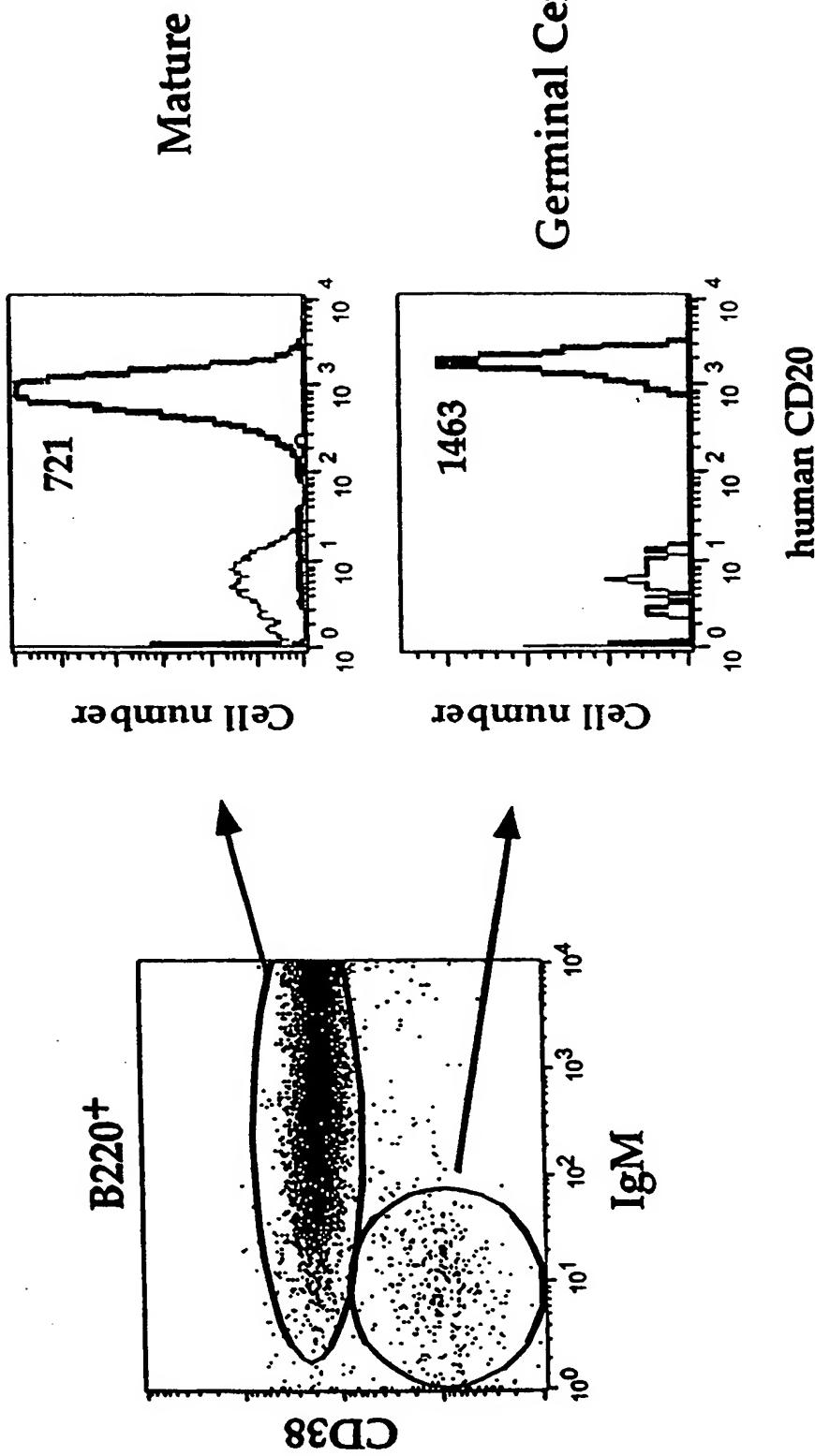
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Effects of anti-CD20 mAb in mice

1.0 mg anti-CD20 mAb   [=50 mg/kg: 3.5 gm for 70 kg man]

Day   -1   1   2   3   4   7   14   21

FACS
Peripheral Blood
Spleen
LN
Bone Marrow
Peyer's Patch
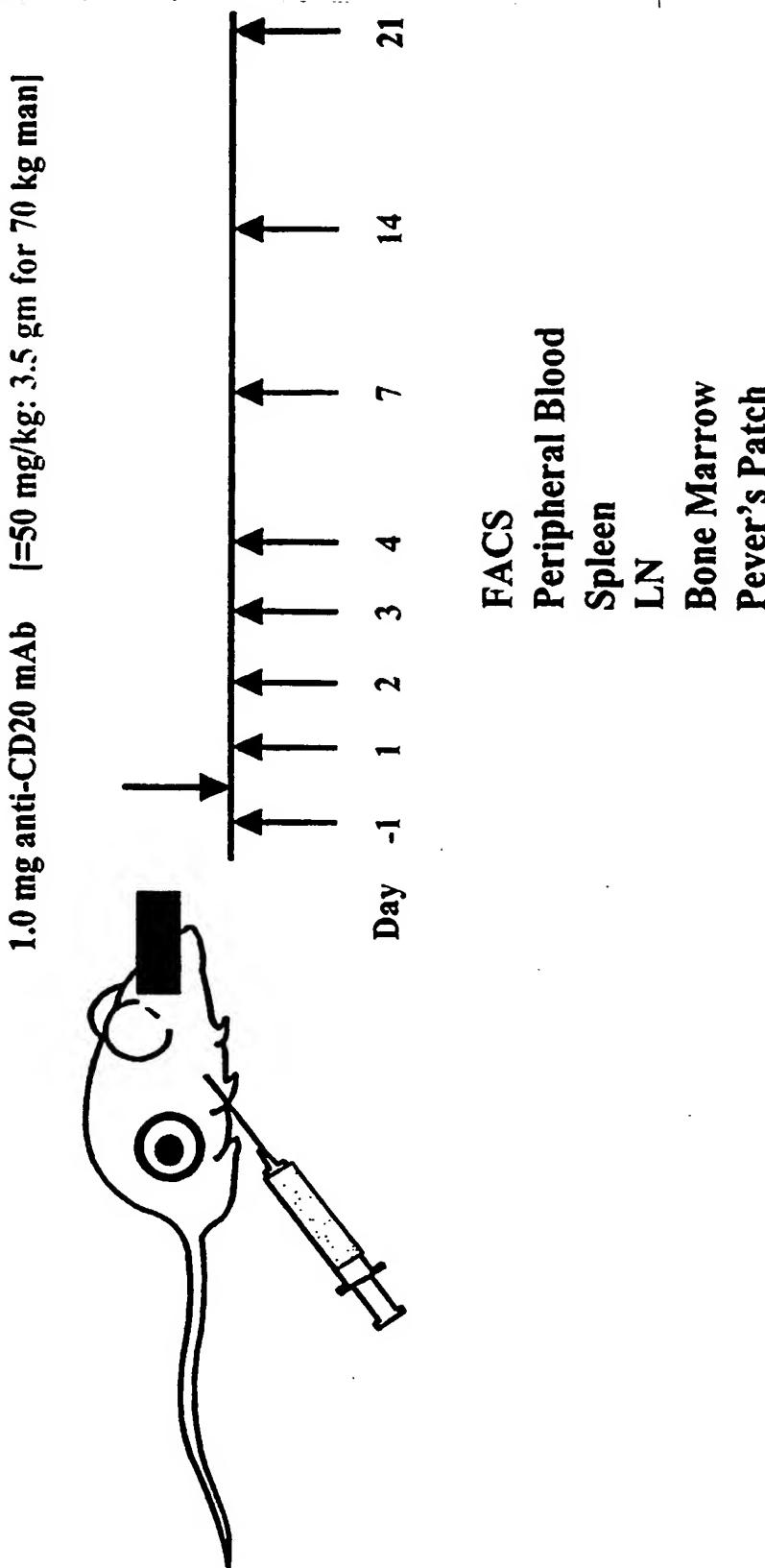
Serum levels of anti-CD20 mAb

Figure 7

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Depletion of peripheral B cells with anti-CD20 mAbs
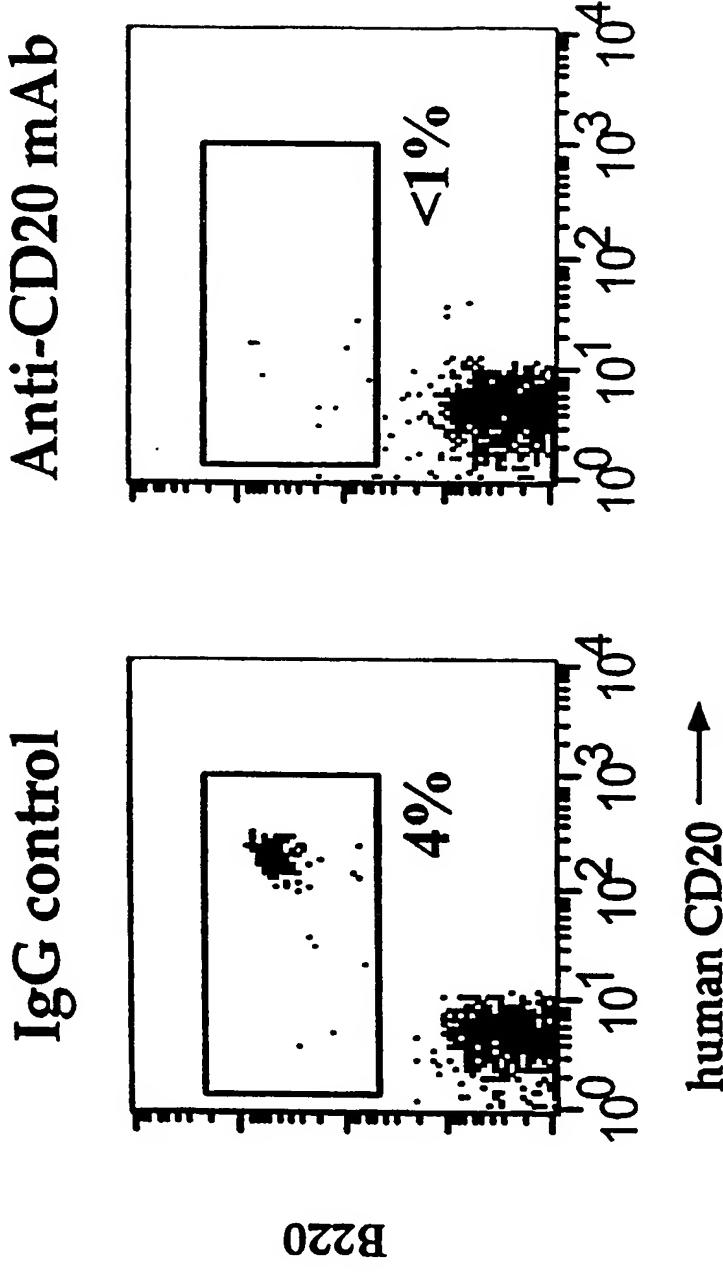
IgG control

Anti-CD20 mAb

4%

<1%

B220

human CD20 ⟶

Figure 8

Depletion of mature peripheral LN B cells by anti-CD20 mAb

Anti-CD20 mAb

<1%

IgG

11%

CD23

CD21

Figure 9

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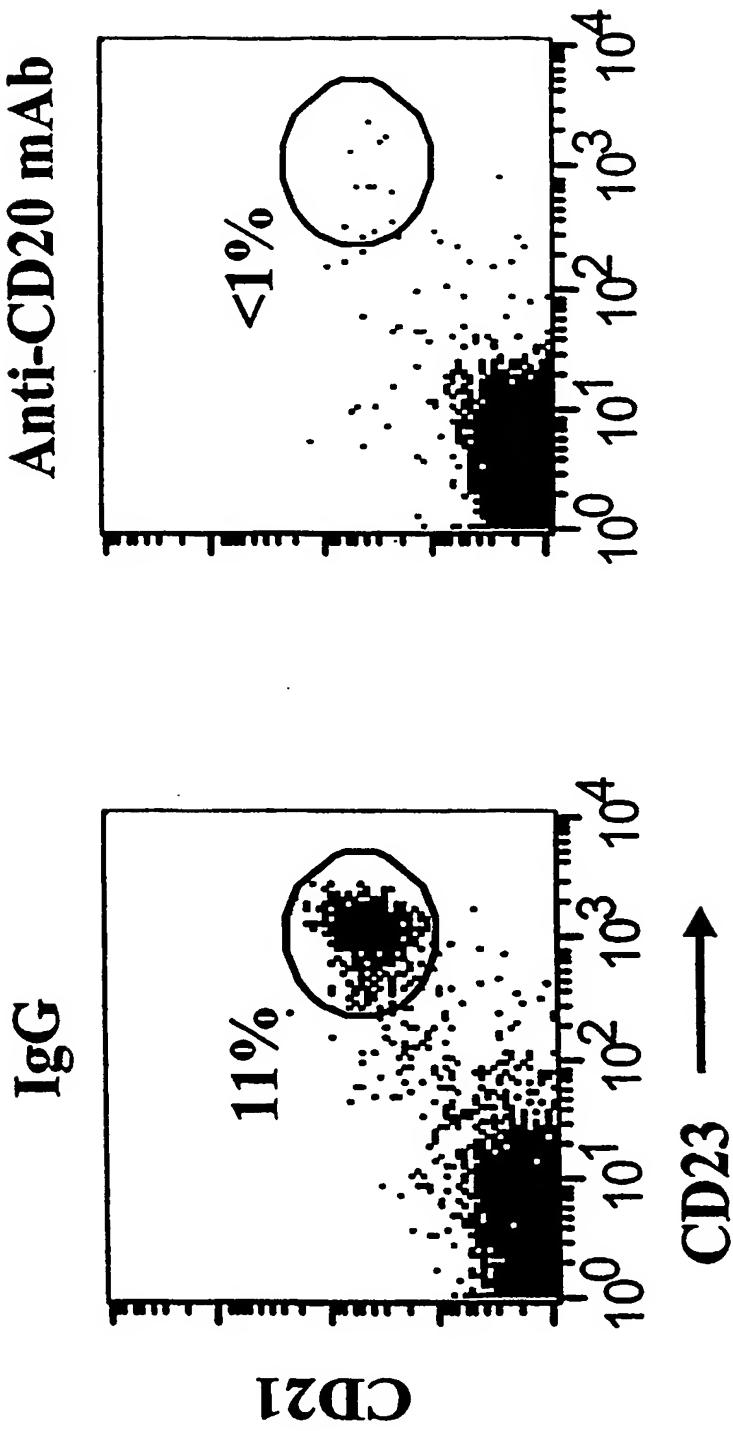
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Depletion of splenic T2 B cells by anti-CD20 mAbs
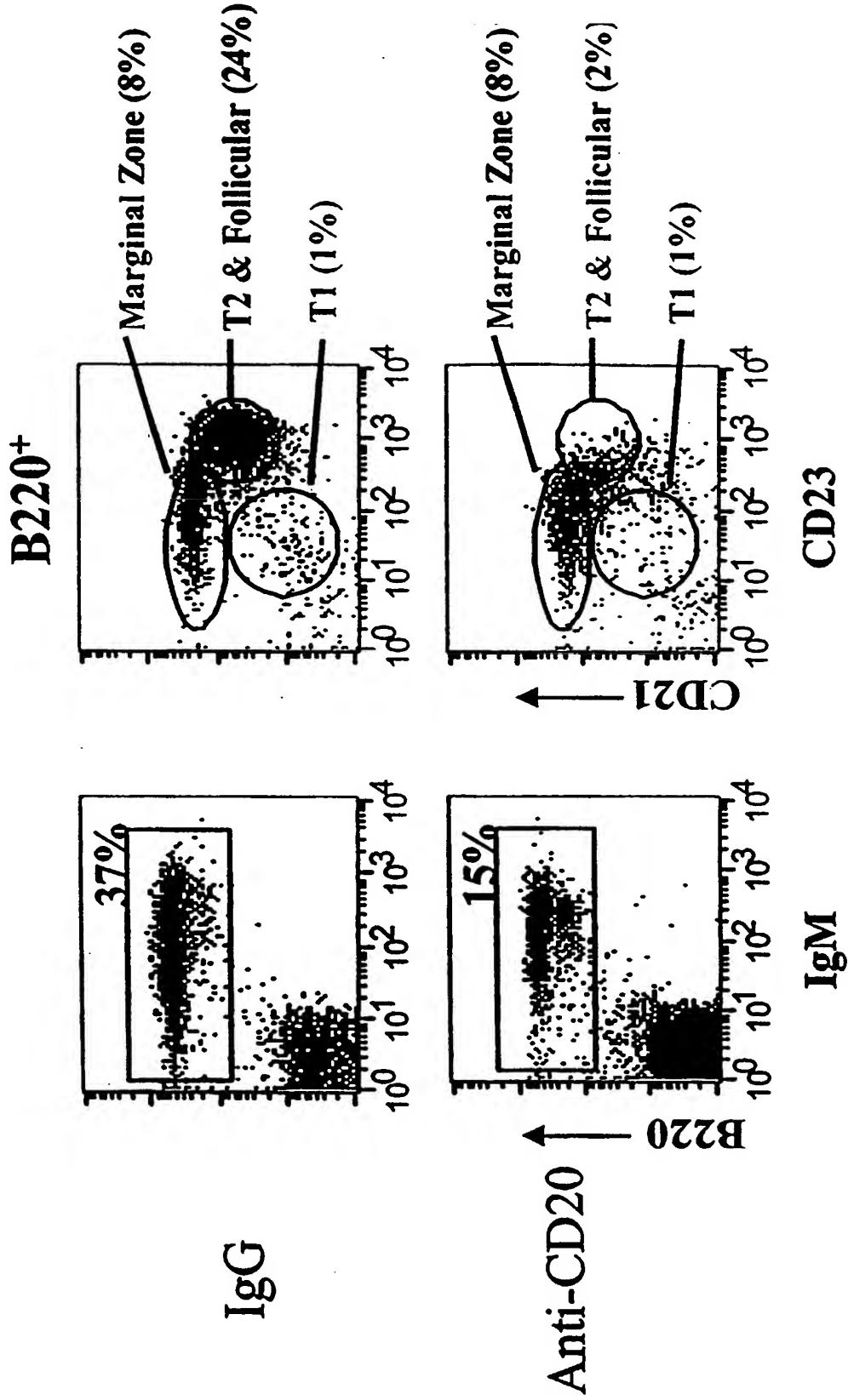
Figure 10

# Depletion of re-circulating mature B cells by anti-CD20 mAbs
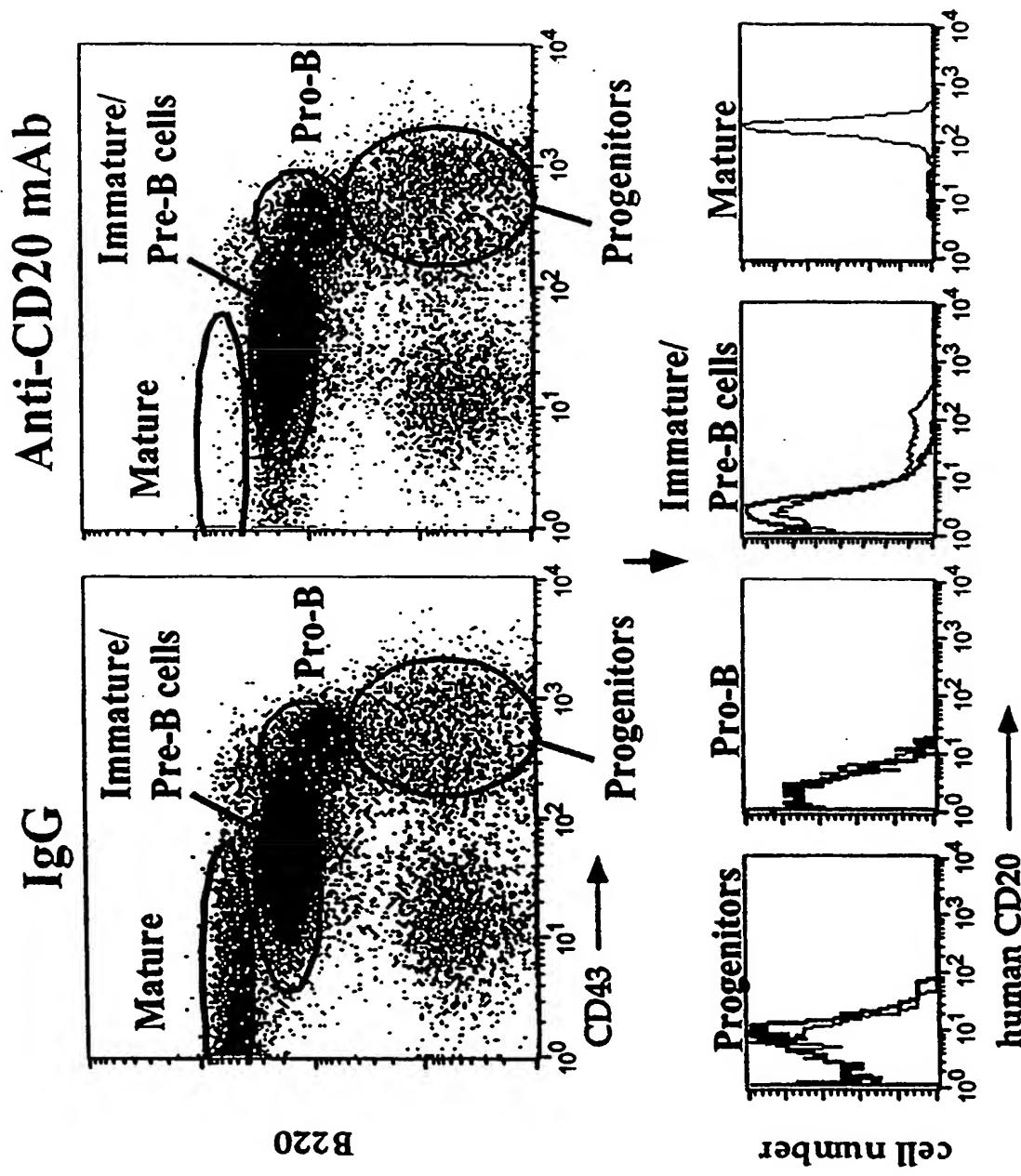
Figure 11

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Resistance of Peyer's Patches Germinal Center B cells to anti-CD20 mAbs

IgG

Anti-CD20 mAb

Figure 12

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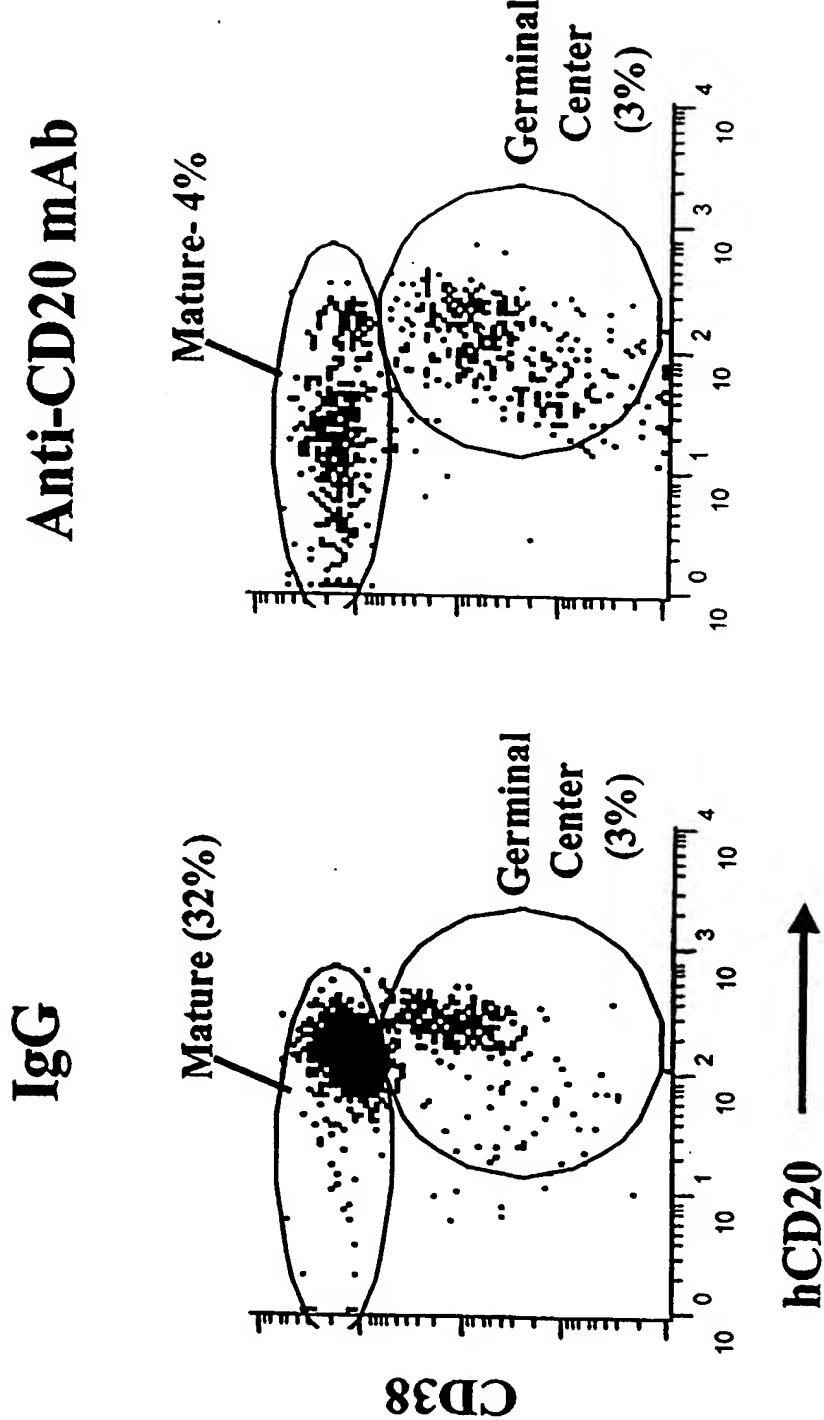
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Depletion & Recovery of B cells following anti-CD20 mAb Rx

Figure 13

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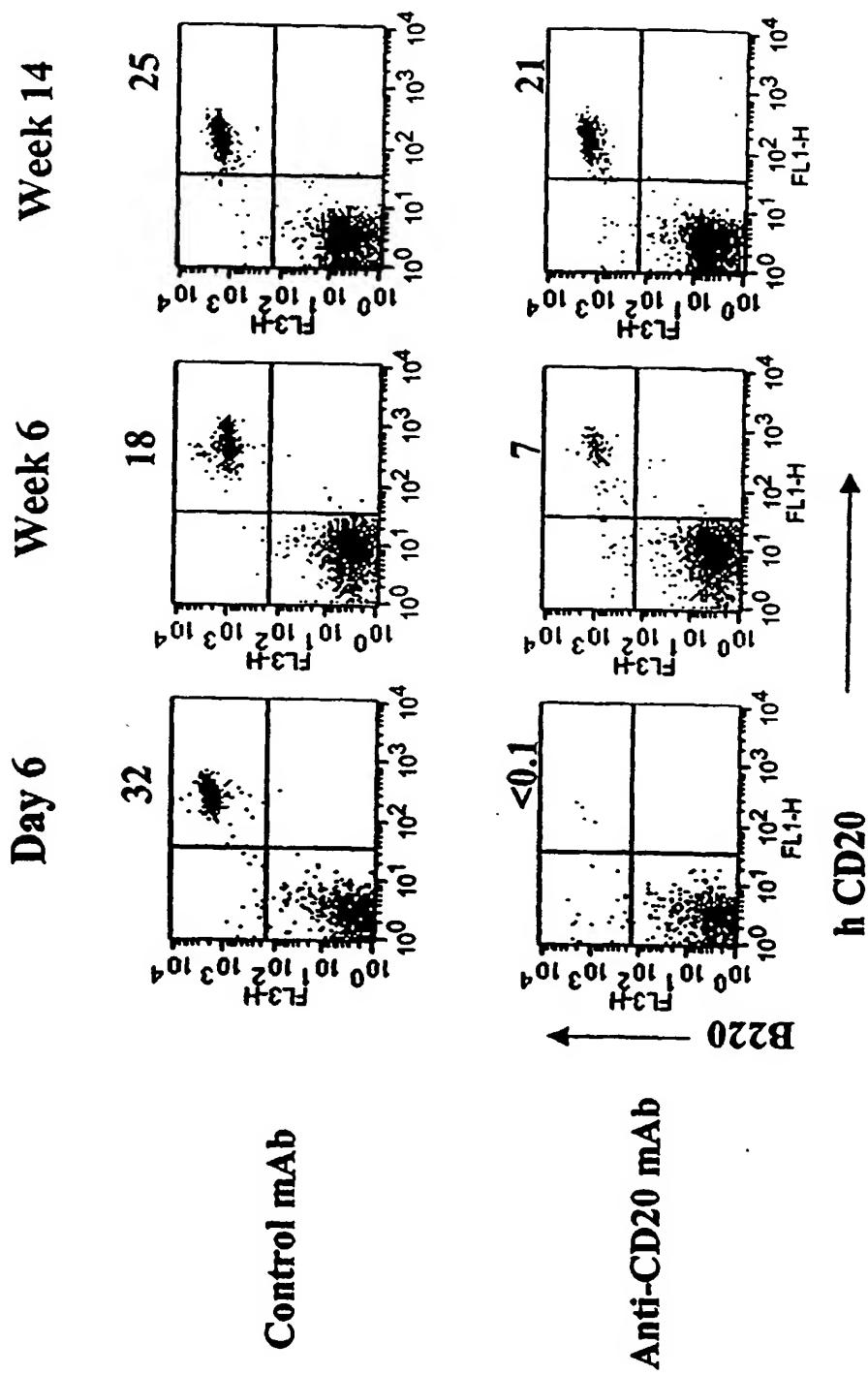
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Resistance of Splenic Germinal Center B cells
to short-term anti-CD20 mAb Rx

Figure 14

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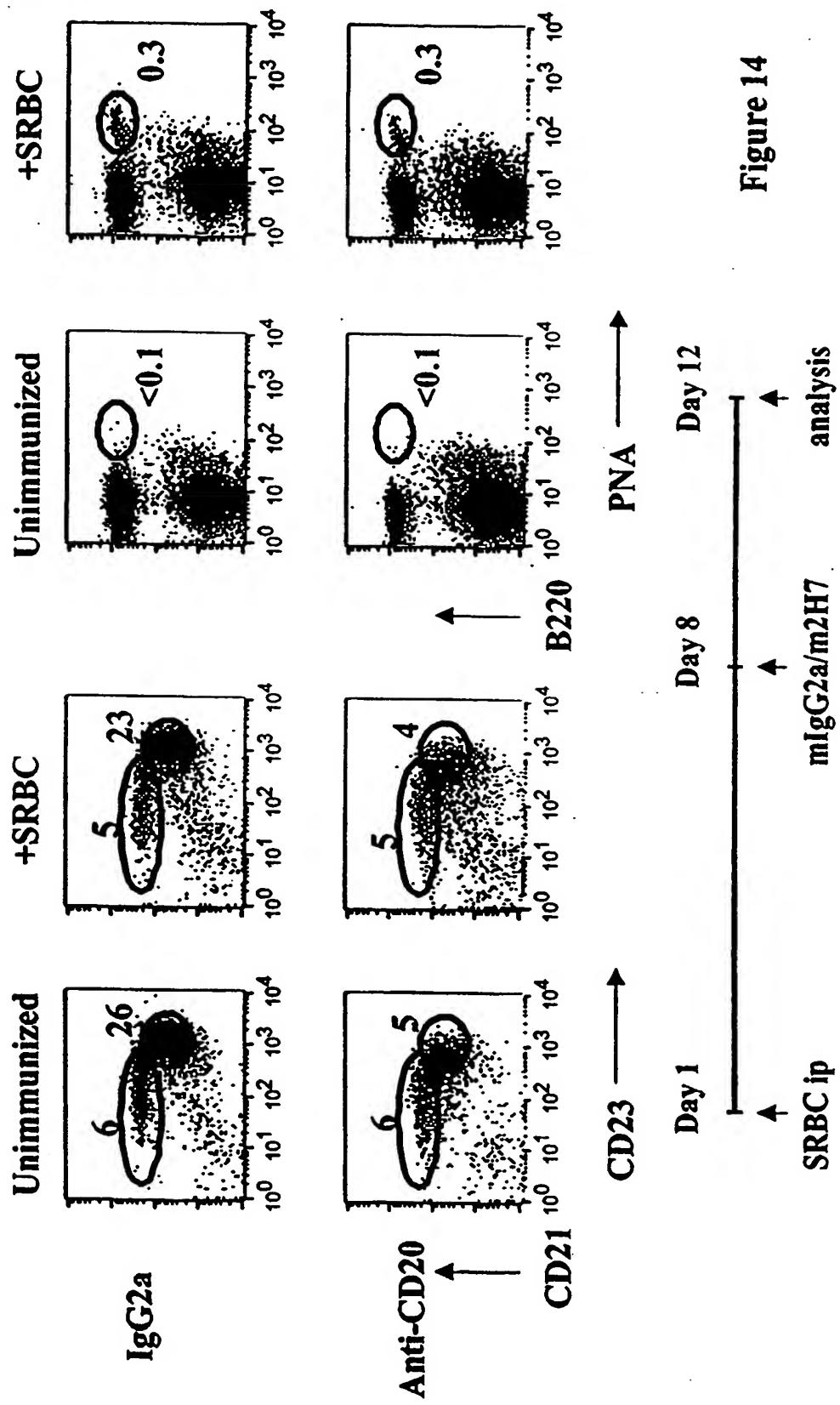
Non-depleted MZ and B1 B cells confer protection to T-Independent antigens

Figure 15

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Figure 16

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In Peyer's Patches

Figure 17

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Plasma cells are not depleted
following long-term anti-CD20 mAb treatment

Figure 18

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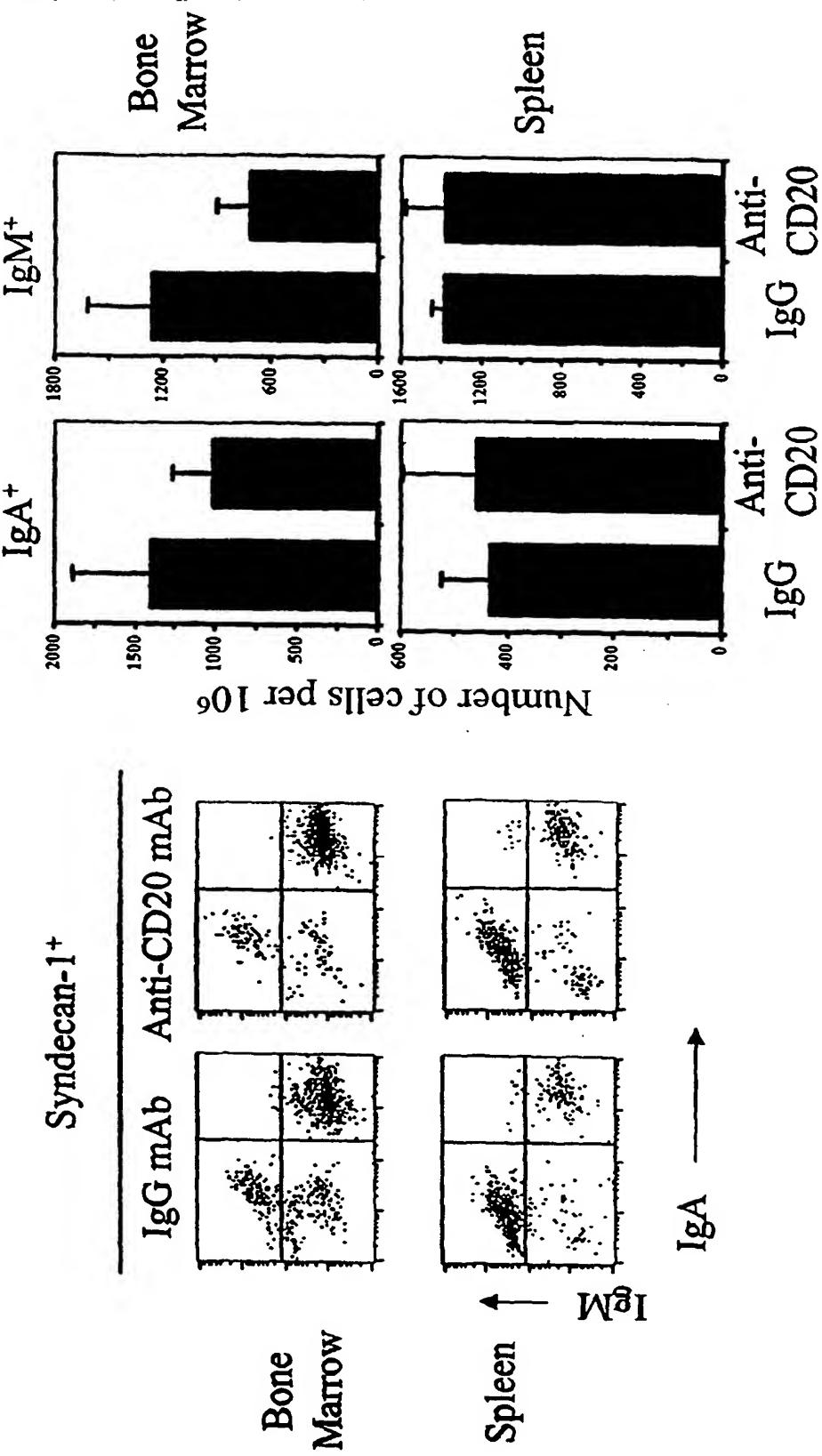
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Depletion of NK cells by PK-136 mAb

Figure 19

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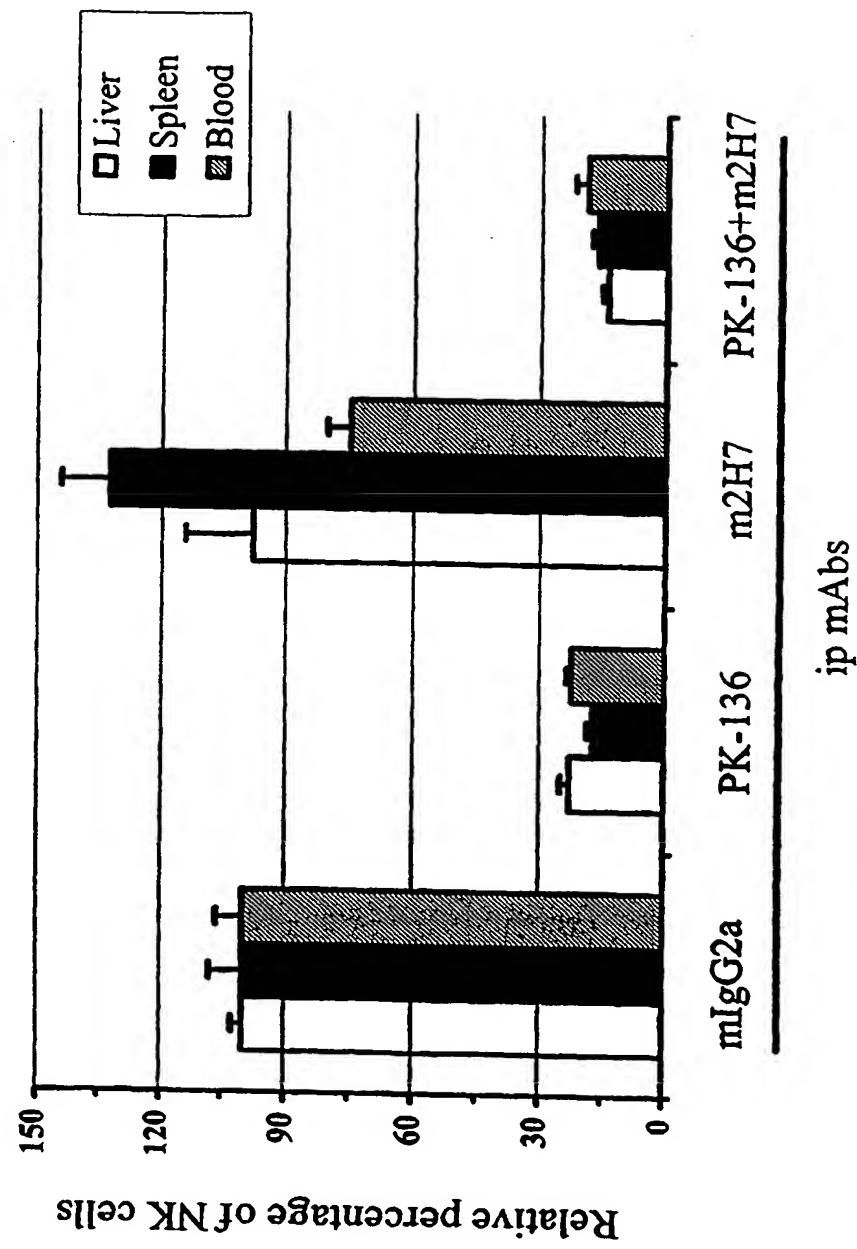
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NK cells play a role in 2H7-mediated B cell depletion
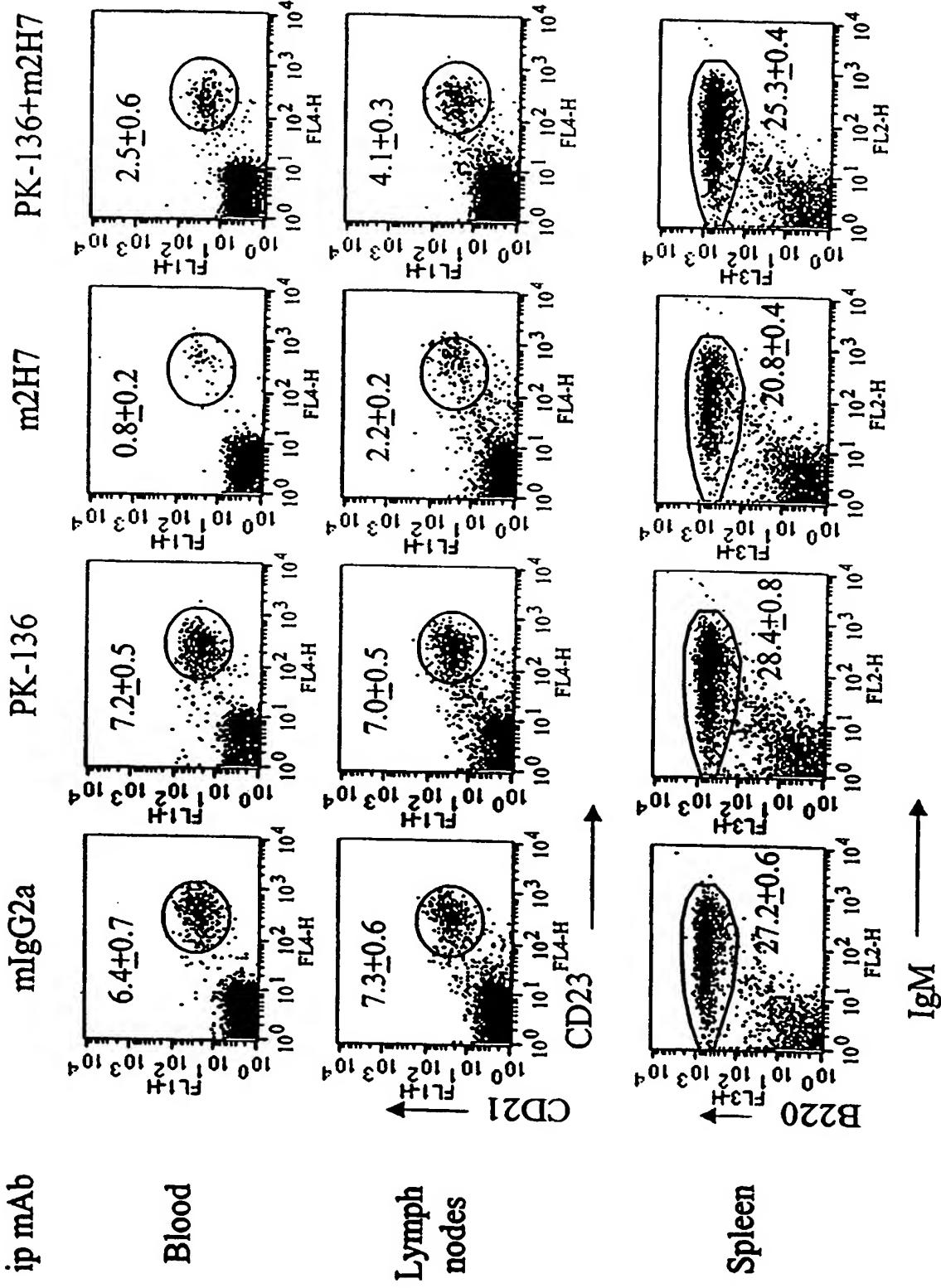
Figure 20

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FIGURE 21A

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSF
FMRESKTLGAVQIMNGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLA
ATEKNSRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTP
YINIYNCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKR
TCSRPKSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETE
TNFPEPPQDQESSPIENDSSP"

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FIGURE 21B

```
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  61 ttgagatttg aggccttgga gactcaggag ttttgagagc aaaAtgacaa cacccagaaa
 121 ttcagtaaat gggactttcc cggcagagcc aatgaaaggc cctattgcta tgcaatctgg
 181 tccaaaacca ctcttcagga ggatgtcttc actggtgggc cccacgcaaa gcttcttcat
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 301 ggggggtctt ctgatgatcc cagcagggat ctatgcaccc atctgtgtga ctgtgtggta
 361 ccctctctgg ggaggcatta tgtatattat ttccggatca ctcctggcag caacggagaa
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 901 aatccaagaa gaggaagaag aagaaacaga gacgaacttt ccagaacctc cccaagatca
 961 ggaatcctca ccaatagaaa atgacagctc tccttaagtg atttcttctg ttttctgttt
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Figure 21C

```
   1 tgttaaacca aagtaattgg agcgaagccc agggtagcag aagctactga tttcctgtca
  61 cctgatgtct atcagcgatt tcatcttcag gcctggacta caccactcac cctcccagtg
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1261 acccaggctg gaatgcagtg gcatgatctc agctcactgc aacctctgcc tcctaggttc
1321 aagcaattct cctgccttag cctcccgagt agctgggatt acaggtgcac accaacatgc
1381 ctggctaatt tttgtatttg tagtagagat ggggttttgc catgttagcc agcccggtct
1441 caaactcctg acctcaggtg atctgcccat cttggcctcc caaagtgctt ggattacagg
1501 catgagccac cgtgcccgac ctagagagcc ttcttgatgt gacttgcaca aggtggcaga
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1681 ggacaagtcc attgaaaata catttttcaa tattaaagca aaagaaaagc atccaggaat
1741 aagaaacaaa gaggacatgc agtcatatat gcaaggtgtc ctctacaaag ataaagaatg
1801 ccccaaaccc agttgtcaag atcactggca gggactcctg ggcccacatg ctcttcctaa
1861 acaacccctc catctccttt ctcagaactc agcagtaggc cttgcctcag atccaaggtc
1921 actcggaaga ggccatgtct accctcaatg acactcatgg aggaaatgct gagagaagca
1981 ttcagatgca tgacacaagg taagactgcc aaaaatcttg ttcttgctct cctcattttg
2041 ttatttgttt tatttttagg agttttgaga gcaaaatgac aacacccaga aattcagtaa
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Figure 21C cont'd

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2161 cactcttcag gaggatgtct tcactggtgg gccccacgca aagcttcttc atgagggaat

2221 ctaagacttt gggggtaagt cagttgcctt ccatcccatg tcgtagggat tctctggctg

2281 acagaagctg atgcggtata ggtcacatac agaattcaat ccaatttgaa gaattgggat

2341 ccaacctgat gtcttcttta tgtctaacac agtgggccaa atcaggggtg catcagagaa

2401 gttatcactt agatcacctc tgggtgatct tatgtcacct tttggttttg gggcttgtat

2461 atgcaggggt tcccccattc ccagttccat ttgccagaat cccaggcata cctgctccct

2521 ggaaatgccc catgtggttg aggaaacaga ttcgaacaag aaaaagacaa aattcttggc

2581 acctccactg cttcctttag gcattcctca cagctccaag tcaggagcca gagcttccaa

2641 ccttgtcttt gcctgctagc agtgatgatt tcagctcatc cactgctgcc tctgttctct

2701 ccccaggctg tccagattat gaatgggctc ttccacattg ccctggggg tcttctgatc

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2821 attatggtga gtaaaagaat agcagccatt tgggaaatgg tgcagacaaa aatgttaaaa

2881 ggctccacag ggatatgcca gattatttct gtgttgaggg aaatatatga gtaggaaata

2941 ttattgggtt aaagtaatta agaagacagg ttgaccaaat tgagtataaa tcccatggtt

3001 gagagtcagt ggtcctgttt catgtgaatt cagagaaagg ggccctgcat ggatctcaca

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3661 caggaagtgt ttggcaagta accatatgtc cttctttccc acatgtcaga gaagtaccta

3721 ttttttcgg ttaaaaactg agacccttaa aaagccatgg tatcacagcc tctcagccct

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4021 agctaagcca tttttggtc gtaacattga acaagtcagt ttacctctat gttcctgagt

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Figure 21C cont'd

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4501 gcaacataaa ataataatca ataatcccat atcattatgg tacttgttat ttatattttc

4561 ctgtttcaac cttttatcat ccctgcaagg tagaacattc acactgatat tctcttacct

4621 atgctaccca aagacatcag ccctaattgt attttggaag atagctgact ggggctgatt

4681 gcagcctatg tcagcaggaa tagatgttgt tactgttgtt gcttctgctt tttttatttc

4741 catttatttg atagtacaga tctagagggt tctatctgaa ccttcccaac ctatacttca

4801 taataccatc ccactaaagt gtgatacaag aaacttcttc actctcttcc ctctacctat

4861 ttatgaaggc agataataaa ctggataata tttatcttca cttattcaac aaacatttat

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5161 tgcatttgga attccctccc agattatgtt ttccaaaggg aaatcaaacc caattaataa

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5341 tttaaaaatg gagagtctga attttattag agctcacaca ccatatatta acatatacaa

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5461 acaatctctg ttcttggtaa gtgttcttgg taagtgtgag attggatttc tctccaggga

5521 ggaaggatga cttgtttatt atgagcatga actctgaatt ccagaccacc tgtgtttgtc

5581 tgcttcaact gattattcat accttacttt ctatcagcaa tacacattaa ccatctgttg

5641 tgtgccaaaa gttgtgttaa gagttagggt tataaagatg ctgtctcctg tactagcagt

5701 tctcacagct attcattact tgtctaaaga attgatctct taatcgttca attatagtca

5761 acaataactt actgaacacc aacaatgttc tttgtgccat tattacattt tcaccttcat

5821 tcttctgttg tttttcaggg cattttgtca gtgatgctga tctttgcctt cttccaggaa

5881 cttgtaatag ctggcatcgt tgagaatgaa tggaaaagaa cgtgctccag acccaaatct

5941 gtaagtagta gcctccagca ccgtggtcaa tgtctgctgc ccttgaagat ttattcagac

6001 ttgagtttta ataaatgact tgataaggat ataagcacct gcaaaaaaat tttggcattt

6061 aaaggcatat aataaatgac ataagtagca taaaaaccag gaggtatttg ataaatgttt

6121 gtggagattg ttgacaaagg tgtcagttgt aaaagtaaag aatggtttgt ttaattttct

6181 gttttagaac atagttctcc tgtcagcaga agaaaaaaaa gaacagacta ttgaaataaa

6241 agaagaagtg gttgggctaa ctgaaacatc ttcccaacca aagaatgaag aagacattga
```

Inventor: CHEN et al.
Docket No.: 11669.0150USW2
Title: TRANSGENIC MICE EXPRESSING HUMAN CD20
Attorney Name: Katherine M. Kowalchyk
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Figure 21C cont'd

6301 aattattcca atccaagaag aggaagaaga agaaacagag acgaactttc cagaacctcc

6361 ccaagatcag gaatcctcac caatagaaaa tgacagctct ccttaa

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FIGURE 21D

Mouse CD20

```
   1 gaattccttt tttttttttt tttttaaaga tttatttatt attatatgta agtacactgt
  61 agctatcttc aagtacttga gatagaagag gccaactgat ctcagctgtg agtggctaat
 121 ttggcccctta agccttggag ccttggagcc ttggagaccc aggcgtttga aaactcaatg
 181 agtggacctt tcccagcaga gcctacaaaa ggtcccctcg ccatgcaacc tgctccaaaa
 241 gtgaacctca aaaggacatc ttcactggtg ggccccacac aaagcttctt catgagggaa
 301 tcaaaggctt tgggggctgt ccaaatcatg aatggcctct tccatattac cctggggggga
 361 ctgctgatga tccccacagg ggtcttcgca cccatctgtt tgagtgtatg gtaccctctc
 421 tggggaggca ttatgtacat tatttcagga tcactcctgg cagctgcagc agaaaaaacc
 481 tccaggaaga gtttggtcaa agcaaaagtg ataatgagct ctctaagcct ctttgctgcc
 541 atttctggaa taattctttc aatcatggac atacttaaca tgacactttc tcattttta
 601 aaaatgagaa gactggagct tattcaaact tccaagccgt atgttgatat ctacgactgt
 661 gaaccatcta attcctcaga gaaaaactcc ccatctacac agtactgtaa cagcattcag
 721 tctgtgttct tgggcattct gtcggcgatg ctgatctctg ccttcttcca gaaacttgtg
 781 acagctggta ttgtggagaa tgagtggaaa agaatgtgta ccagatccaa atctaatgtg
 841 gttctgctgt cagctggaga aaaaaatgag cagacgatta aaatgaaaga agaaatcatt
 901 gagctaagtg gagtatcttc ccaaccaaag aatgaagagg aaattgaaat tattccagtg
 961 caggaggaag aagaagaaga agcagaaata aattttccag cacctcccca agagcaggaa
1021 tccttgccag tggaaaatga gatcgctcct taaactcttt tcttttctaa gcattattgt
1081 ttagagagct tccaagacac atagttaccc tcatctcttg tggccttcca caatctattc
1141 tccatatttt cacagcttaa ctttgcatag agaagccaca tctagctctc cttcacattt
1201 gaagaatgca gtgattataa aagattgtct tttgccttgc ttagggagtc ttacactggc
1261 agaaacgctg aagaatccaa ttctcattca ccttttcctt ggatgtgtgt ctcagtagtg
1321 gtaatggttt ttccgcattt cctccatcag cagttacagc agaaggagtc agagagttca
1381 gggaattc
```

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Figure 22

Human CD20 expression in peripheral blood

Isotype control

hCD20 Tg+ mouse

Human blood

1311
3480
9

hCD20

Cell Number

*: gated on B cells